Title: **MICROBIAL SAMPLE PRESERVATION**

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1.0 OBJECTIVE

The purpose of this method is to preserve microbial samples for bacterial enumeration or protist taxonomic identification/quantification. This method specifically uses glutaraldehyde as the fixative. Other preservatives such as formalin and Lugol's solution may be preferred, depending on the application. Please refer to Kemp (1993) for additional guidance on microbial sampling and analysis.

2.0 HEALTH AND SAFETY

Personnel should wear lab coats, safety goggles and chemical resistant gloves when handling chemical preservatives. Sample preservation should be performed in a chemical fume hood.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Personnel should not perform this method until training by experienced individuals is complete.

4.0 REQUIRED AND RECOMMENDED MATERIALS

50% wt/wt glutaraldehyde (GTA) (use phosphate buffered GTA for freshwater samples)
sterile disposable glass pipets (1 and 10 ml)
pipet bulbs
15 mL polystyrene centrifuge tubes
rack to hold centrifuge tubes
aluminum foil

refrigerator
vortex mixer

5.0 PROCEDURE

5.1 Sample preservation

- Collect samples in sterile containers. Keep cool and dark during transport to lab.

- Samples should be processed immediately upon receipt (no more than 24 hours after collection). Samples are homogenized by gently stirring the contents and aliquots are removed for various microbial analyses (see appropriate SOPs for volume to preserve, usually 10 mL from PFU samples).

- Preserve samples to a final volume of 2% GTA by adding sample to a labeled, sterile 15 mL screw-top centrifuge tube already containing cold fixative. For example, add 9.6 mL of sample to 0.4 mL of 50% GTA. Vortex lightly or invert several times to mix.

5.2 Sample storage

- Make sure the tubes are tightly capped; cover samples with aluminum foil and store in the refrigerator. Fixed samples may be stored in the refrigerated (4 °C in the dark) for up to three weeks without significant changes in bacteria and for 2-3 months without significant changes in protists.

6.0 QUALITY CONTROL/QUALITY ASSURANCE

If preserving samples for bacterial enumeration, it is advisable to sterile filter (0.2 um pore size) the GTA prior to use.

7.0 REFERENCES

Kemp PF (ed). 1993. Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, FL.